

E1

The unamplified SGBAF-1 cDNA library (10^6 recombinants) was plated on E.coli K12 PLK-F' (Stratagene) at a density of 10^5 plaques per 15 cm dish and lifts taken in duplicate onto nitrocellulose membranes (Millipore). For screening, filters were prehybridized for at least 1 h at 42°C in 6 x SSPE, 0.5% SDS, 10 x Denhardt's solution, $100\text{ }\mu\text{g ml}^{-1}$ denatured sonicated herring sperm DNA (Sigma). Hybridization was carried out in the same solution containing 10 ng ml^{-1} radiolabelled oligonucleotide. Oligonucleotides used were: Peptide N (MDWIFHT) (SEQ ID NO: 11) 5'-AA(G/A)ATTGGA(T/C)TGGAT(C/T/A)TT(T/C)CA(T/C)AC-3' (SEQ ID NO: 12); Peptide J(D D G Q L F H I D F G H F) (SEQ ID NO: 13); 5'-GATGATGGCCA(G/A)CTGTT(T/C)CA(T/C)AT(T/A)GA(T/C)TTTGGCCA(T/C)T T (SEQ ID NO: 14). Oligonucleotides were labelled with ^{32}P at the 5' end in a $20\text{ }\mu\text{l}$ reaction containing 100 ng oligonucleotide, 1 x kinase buffer (Promega), 0.1 mM spermidine, 5 mM dithiothreitol, $100\text{ }\mu\text{Ci}$ [$\gamma\text{-}^{32}\text{P}$]ATP (5000 Ci mmol^{-1} , Amersham) and $2\text{ }\mu\text{l}$ (200 U) 54 polynucleotide kinase (Amersham). Filters

Page 39, lines 18-38: replace by the following:

E2

RACE PCR was carried out essentially as published previously (Frohman, et al., 1988; Harvey and Garlison, 1991). Briefly, first strand cDNA primed with random hexamers (Amersham) was synthesized from $1\text{ }\mu\text{g}$ of SGBAF-1 cell mRNA using the Stratagene first strand cDNA synthesis kit. First strand cDNA was isolated by isopropanol precipitation and tailed with oligo-dA using terminal deoxynucleotidyl transferase (BRL). PCR was performed using oligo 2224 (5'-AATTCACACACTGGCATGCCGAT) (SEQ ID NO: 15) and adaptor-dT (5'-GACTCGAGTCGACATCGATTTTTTTTTTTTTTTTTT) (SEQ ID NO: 16) as primers using a Perkin Elmer/Cetus Tap polymerase PCR kit (conditions: 94°C 1 min, 35°C 1 min, 72°C 2 min, 30 cycles). Products were fractionated on a 1.5% low melting point agarose gel and visualized by staining with ethidium bromide. The gel was sliced into 6 bands (size range 150-2000 bp) and DNA isolated from each gel slice. A further round of PCR 2 was performed on this DNA using oligonucleotide 2280 (5' -TTTAAGCTTAGGCATTCTAAAGTCACTATCATCCC) (SEQ ID NO: 17) and adaptor (5'-GACTCGAGTCGACATCGA) (SEQ ID NO: 18) as primers (conditions: 94°C 1 min, 56°C 1 min, 72°C min, 356 cycles. Products were fractionated on an agarose gel and visualized by staining with ethidium.

Page 41, lines 12-35: replace by the following:

For p85 α 125 ng of poly (A) $^+$ RNA was reverse transcribed with 2.5 units rTth DNA polymerase (Perkin-Elmer-Cetus) at 70°C for 10 min in a $10\text{ }\mu\text{l}$ reaction containing 10 mM Tris-HCl (pH 8.3), 90 mM KCl, 1 mM MnCl, 0.5 mM dNPT mixture and $1.2\text{ }\mu\text{M}$ antisense primer (5'-CAGGCCTGGCTTCCTGT) (SEQ ID NO: 19). For DNA polymerization the reaction volume was adjusted to $50\text{ }\mu\text{l}$ by adding a single mix giving the following final concentrations: 5% (v/v) glycerol, 10 mM Tris-HCl (pH 8.3), 100 mM KCl, 0.75 mM EGTA, 0.05% (v/v) Tween 20, 2 mM MgCl $_2$, $0.24\text{ }\mu\text{M}$ sense primer (5'-AACCAGGCTCAACTGTT)

(SEQ ID NO: 20). PCR was then performed under the following reaction conditions: 92°C 1 min, 58°C 1 min, 72°C 1 min for 25 cycles on a Perkin Elmer-Cetus DNA thermal cycler.

Conditions for p110 were similar except concentration of the antisense primer (5'-TGCTGTAAATTCTAAATGCTG) (SEQ ID NO: 21) was increased to 4.8 µM during the reverse transcription step. DNA polymerisation conditions were the same except the final MgCl₂ concentration was increased to 2.5 mM and both primers (sense primer = 5'-GTATTTTCATGAAACAAATGA) (SEQ ID NO: 22) were present at a final concentration of 0.96 µM. Taq DNA polymerase (Promega) was also added at 0.03 U µl⁻¹. PCR was performed as follows: 92°C 30 sec, 54°C 5 sec, 72°C 30 sec for 35 cycles. 20 µl of each reaction was run on a 3% agarose gel (Maniatis, et al. 1982) and visualised by staining with ethidium bromide.

Page 42, lines 1-10: replace by the following:

peptide CKMDWIFHTIKQHALN (SEQ ID NO: 23) was synthesized by Fmoc chemistry and purified by HPLC. It was then coupled to KLH using glutaraldehyde, and injected into the lymph nodes of rabbits using methods described in Kypta, R M et al., (1990), Cell 62, 481-492. Positive antisera as determined by enzyme-linked immunoassay were affinity purified on specific peptide-Actigel affinity columns. Anti-p85α (Otsu, et al., 1991) and anti CSF-1 receptor (Ashmun, et al., 1989) antisera are previously documented. Immunoprecipitations were carried out as described in Otsu, et al., 1991.

Page 52, lines 26-38 replace by the following:

The human cDNA was isolated from a cDNA library, made from mRNA isolated from the human cell line KG1a using standard techniques. The probe was a partial cDNA from the second half of the bovine p110 cDNA. The probe was labelled with ³²P and hybridised overnight to the library filters at 65°C in 1M NaPi, 7% SDS buffer. The filters were washed in 2xSSC at 50°C, and exposed to X-ray film at -70°C. The nucleotide sequence is shown in Figure 16 together with the corresponding amino acid sequence. The human p110 sequence has 95% homology to the bovine p119 sequence at the DNA level and is 98% identical at the protein level (Figures 17 and 18). The protein sequence is shown in Figure 19. Primers (357) AAG GAT CAG AAC AAT GCC T (SEQ ID NO: 24) and (416) AGG CTT TCT TTA GCC ATC A (SEQ ID NO: 25) were

Page 53, lines 5-23: replace by the following:

Two novel cDNAs related to p110 have been cloned. Degenerate primers were designed to conserved sequences between human p110 and the related yeast gene VPS34 (Sense (GDDLQRD) (SEQ ID NO: 26) 5' GGN GAT/C GAT/C T/C TA/G CGN CAA/G GA-3 (SEQ ID NO: 27) antisense (FHIDFGHF) (SEQ ID NO: 28) 5' A/GAA A/GTG ICC A/GAA A/GTC A/G/TAT A/GTG A/GAA-3) (SEQ ID NO: 29). These were used in RT-PCR reactions using mRNA from the